Arch Gynecol Obstet (2017) 295:511–518
DOI 10.1007/s00404-016-4270-z

Lymphocyte immunotherapy in the treatment of recurrent miscarriage: systematic review and meta-analysis

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Received: 18 October 2016 / Accepted: 9 December 2016 / Published online: 21 December 2016

Abstract
Purpose Recurrent miscarriage (RM) affects up to 2–3% of couples of reproductive age. There are several causes for this condition, including immunologic. The embryo is considered an allograft, subject to the rejection mechanisms of the maternal immune system. Immunotherapy involving immunization with lymphocytes is considered in cases of idiopathic RM. However, there is still no consensus regarding the efficacy and safety of this therapy.
Methods This systematic review and meta-analysis evaluated the data available in the literature regarding the efficacy and safety of the use of immunotherapy with lymphocytes in couples with history of RM. Searches in PubMed/Medline, SCOPUS, and Cochrane Library databases were conducted, using the following keywords: "recurrent miscarriage," "lymphocyte immunotherapy," and "meta-analysis." Statistical analyses were performed using Review Manager 5.3 (RevMan), version 5.3.
Results Six published meta-analysis were retrieved; two found no improvements in the rate of live births after the use of immunization with lymphocytes in the treatment of RM, and four found a beneficial effect of the use of immunotherapy with lymphocytes in cases of RM, with significant improvements in the rate of live births.
Conclusion Data available in the literature supports the efficacy and safety of immunotherapy with lymphocytes in cases of RM without an identified cause.

Keywords Lymphocyte immunotherapy · Recurrent miscarriage · Systematic review

Introduction

Recurrent miscarriage (RM) has historically been defined as three or more consecutive pregnancy losses [1]. In 2009, the American Society for Reproductive Medicine (ASRM) defined this condition as the occurrence of two or more consecutive miscarriages. RM affects up to 5% of couples of reproductive age. The risk increases with a greater number of previous miscarriages. Couple evaluation and early treatment of RM are essential for a successful pregnancy [2, 3].

Several risk factors have been linked with RM; the most studied are genetic disorders, congenital or acquired alterations in the uterine anatomy, hormonal diseases, obesity, and antiphospholipid syndrome. Other causes of RM are immunological disorders (auto- and alloimmune), hereditary thrombophilia, infection, environmental factors, and causes related to male factors, or a combination of these factors [3].

In 1953, Medawar postulated that the fetus is considered as an allograft by the mother, and the absence of maternal

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immune response would allow for embryonic implantation [4]. In 1966, Clark and Kirby suggested that an antigenic disparity between the embryo and the mother is beneficial for gestation [5]. Since then, the theoretical basis for the role of the immune system in the gestational process, from implantation to birth, has been well established.

The first alloimmune mechanism proposed as a cause of RM suggested that the compatibility of human leukocyte antigens (HLA) between father and mother would cause failure in the production of anti-paternal cytotoxic antibodies, anti-idiotypic antibodies (Ab2), and mixed lymphocyte reaction blocking antibodies (MLR-Bf), thus leading to pregnancy loss [6]. Later, other alloimmune mechanisms have been described as being responsible for RM, including (1) natural killer cells (NK) hyperactivity [7]; (2) imbalance of the T-helper 1 (Th1) and Th2 immune response, with a predominance of Th1 response [8]; and (3) low concentration of regulatory T-cells (Treg cells), CD4+ CD25+ FoxP3+ [9].

The reproductive immunology clinical practice is still limited and criticized due to the lack of robust scientific evidence. However, despite these difficulties, many researchers believe that immune therapies are a viable alternative for improving the rates of live births in cases of RM and implantation failure [10].

In 2012, Kwak-Kim et al. evaluated the protocols of 217 assisted reproduction centers worldwide for assessing the immunological factors in RM. Immunological assessment is routinely performed in 69% of the evaluated centers [10]. These studies include NK alterations in peripheral (NKP) and uterine blood (NKU), predominance of maternal Th-1 immune response, HLA compatibility, and reduced Treg cells (CD4+ CD25+ FoxP3+) [10].

Based on different immunological mechanisms of fetal allorejection, several immunotherapies have been proposed to assist the process of embryo implantation and pregnancy maintenance [3]. Immunization with lymphocytes is the most studied immunologic treatment for RM [11–18]. Other immunotherapies have also been studied, such as intravenous human immunoglobulin, steroids, anti-TNF drugs, intralipid, and immunosuppressant drugs [3, 19, 20].

Studies from the early 1970s observed improvements in the results of kidney transplants in patients undergoing blood transfusions [21]. Based on the theory by Opelz et al. [21] of immunosuppression as an anti-rejection mechanism, in the early 1980s, Taylor and Faulk described the successful pregnancy of three patients with the history of RM treated with leukocyte-rich plasma from an unrelated donor [11]. This therapeutic approach was perfected by Mowbray and Alan Beer [6, 12]. The proposed mechanisms of action are production of anti-paternal cyto-toxic antibodies, anti-idiotypic antibodies (Ab2) and mixed lymphocyte reaction blocking antibodies (MLR-Bf), reduced NK cell activity, improved Th-1/Th-2 balance with Th-2 predominance, and improved Treg cell profile [13–16].

Since the first double-blind, randomized study published in 1985 by Mowbray et al., the international literature has been discussing the efficacy of immunotherapy with lymphocytes in the treatment of RM [12]. Different factors hinder the strength of the evidence on the issue and need to be better defined, such as (1) studies with an inappropriate number of participants, due to the high cost of research and low incidence of RM; (2) difficulty in defining an ideal criteria for selecting couples to undergo treatment; (3) different treatment protocols with various forms of preparation and different lymphocyte concentrations per immunization dose, with applications only before or before and during pregnancy, and no standardization regarding the administration route (intravenous, intramuscular, subcutaneous, or intradermal); and (4) standardization of pre-pregnancy immunotherapy control [17, 18, 22].

Methods

This systematic review and meta-analysis, based on the PRISMA Statement recommendations, aimed to evaluate meta-analyses on the use of immunotherapy with lymphocytes for the treatment of couples with RM history that had been published as of the time of the research in the medical literature. Searches were carried out in the PubMed/Medline, Scopus, and Cochrane Library databases, using the following key words: "recurrent miscarriage," "lymphocyte immunotherapy," and "meta-analysis." Data related to the efficacy and safety of immunotherapy with lymphocytes from meta-analyses published until the submission of the present review were analyzed. All original statistical analyses were redone, and new analyses were performed using Review Manager (RevMan) [Computer program], Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration).

Results and discussion

Currently, the medical literature features several studies, with the highest level of scientific evidence, on the use of immunization with lymphocytes in the treatment of couples with RM; there are six meta-analyses [20, 23–27] and dozens of randomized clinical trials [28–43]. Among the published meta-analyses, two observed no improvement in the rate of live births [20, 23] after the use of immunization with lymphocytes in the treatment of RM, while four found a beneficial effect of the use of immunotherapy with

lymphocytes in RM cases, with significant improvement in the rate of live births [24–27].

Efficacy of immunotherapy with lymphocytes

The first meta-analysis was published in 1993, by Fraser et al., who evaluated the effect of immunotherapy with lymphocytes or infusion of trophoblast membrane in four randomized trials and did not detect an improvement in the rate of live births [23].

In 1991, during the 11th Annual Meeting of the American Society for Reproductive Immunology, the Ethics Committee for Immunotherapy of that society, aiming to increase the sample size and standardize the treatment protocol, organized a multicenter study and meta-analysis to assess the efficacy of immunotherapy with lymphocytes in women with RM. The Recurrent Miscarriage Immunotherapy Trialist Group published the results of their meta-analysis in 1994, evaluating data from 15 centers, including nine randomized trials (seven of which were double blind) that were independently assessed by two separate data analysis teams to ensure that the conclusions were robust. One team also compared randomized trials with the results of six non-randomized controlled studies to test for bias in non-randomized trials [25].

The group concluded that immunotherapy with lymphocytes improved the rate of live births in patients with the history of RM (three or more consecutive miscarriages), with no more than one live birth with any partner [odds ratio (OR) 1.16, confidence interval (CI) 1.04–1.34], or no more than one live birth with the current partner (OR 1.21, CI 1.04–1.37). A significant improvement in the rate of live births was observed when the patients had antibodies against the lymphocytes of their spouses before pregnancy (RR 1.17, CI 1.06–1.27). However, there was a worsening in the rate of live births among patients with autoimmune disorders (positive ANA and/or antiphospholipid antibodies) or who underwent intravenous immunotherapy (RR 0.79, CI 0.66–0.91) [25].

In 2001, the Cochrane Library published a meta-analysis that assessed the different forms of immunologic treatment for cases of RM, including immunization with lymphocytes. The last update of this meta-analysis, in 2014, included 12 studies that performed immunotherapy with paternal lymphocytes, totaling 641 participants, 316 treated women and 325 in the placebo group. The treatment effect on the live birth rate was not significant, with OR of 1.22 and CI of 0.89–1.69 (Fig. 1) [20]. In this same Cochrane meta-analysis, no improvement in the rate of live births was observed when assessing the use of immunotherapy with unrelated donor lymphocytes, with OR of 1.39 and CI of 0.68–2.82 [20].

Christiansen et al., in their original study, performed immunotherapy with unrelated donor lymphocytes. The Cochrane meta-analysis included the results of Christiansen et al.; however, their data from the group treated with paternal lymphocytes are unpublished, and the data used in the analysis of the immunotherapy with unrelated donor lymphocytes are different from those presented in the original article [6, 20–35]. During this systematic review, the authors tried to contact the editorial staff of the Cochrane Library, via email, for clarification on this issue; however, no response was received as of the date of submission of this article for publication.

Numerous researchers in this field criticize the results presented in the Cochrane meta-analysis [17, 18, 22, 26]. The main criticism relates to the inclusion of the results by Ober et al. [43], the only study published to date that observed a negative effect of immunotherapy with lymphocytes on the rate of live births. Ober et al. prepared the concentrate of paternal lymphocytes using blood that had been stored for several hours at a temperature between 1 and 6 °C, increasing the interval between the collection of the blood of the spouse and application of immunization [43]. Clark et al. demonstrated the importance of an adequate number of CD200+ cells for increasing the immunomodulatory effect in immunotherapy with lymphocytes; they also demonstrated that storing total blood at low temperatures reduces the CD200+ cell count [44]. Clark et al. suggested that a new miscarriage in patients undergoing immunotherapy with lymphocytes would be due to an embryonic genetic alteration, undiagnosed autoimmune disease, or immunotherapy conducted with an insufficient number of paternal CD200+ cells [22].

Furthermore, Ober et al. discontinued the study before reaching the initially expected number of participants and did not exclude patients with autoimmune disorders (positive ANA test), which adversely impacts the results of immunotherapy with lymphocytes [43]. Other criticisms of the study by Ober et al. are the lack of post-immunization control of immunomodulation, generated by immunotherapy with lymphocytes, before allowing the couple to attempt a new pregnancy; and different immunotherapy administration routes (intradermal, subcutaneous, and intravenous), number of doses, and lymphocytes concentration [17, 18, 22, 26].

A new analysis of the publications included in the Cochrane Library meta-analysis [20], excluding the data from Ober et al. [43], indicated a significant improvement in the rate of live births in couples who underwent immunotherapy with lymphocytes, OR 1.63, with CI of 1.13–2.35 (Test for overall effect: $Z = 2.60$, $P = 0.009$) (Fig. 2). The withdrawal of the results by Ober et al. allowed for a greater homogeneity of the sample, when compared with the original Cochrane analysis; heterogeneity: $\chi^2 = 13.88$, $df = 10$ ($P = 0.18$); $I^2 = 28\%$ (Fig. 3).

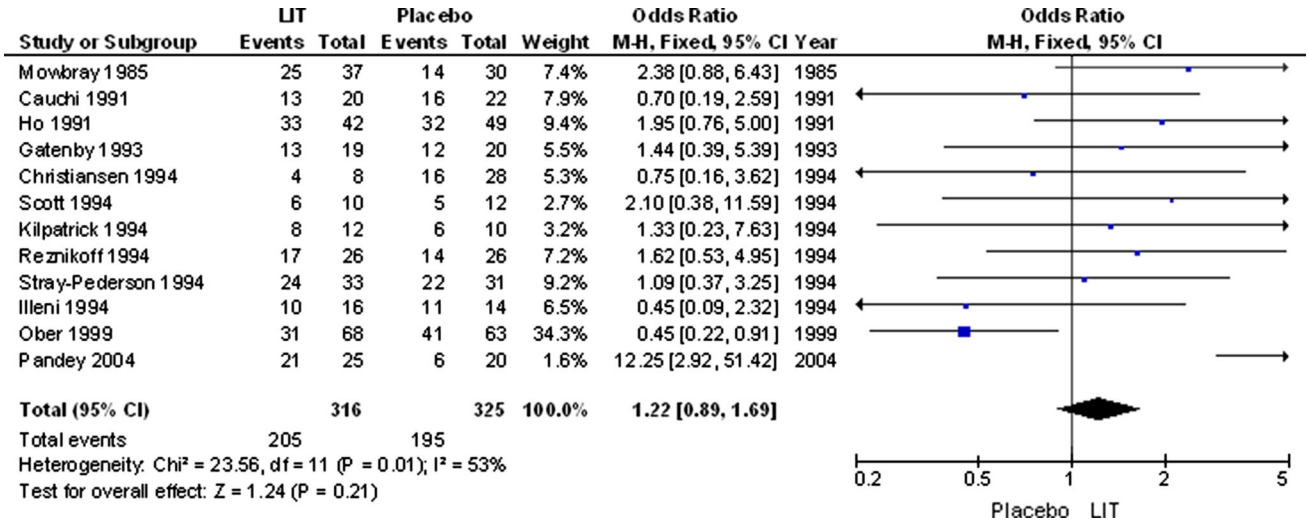

Fig. 1 Statistics from the Cochrane meta-analysis on the effect of immunotherapy with lymphocytes in cases of RM, with the original data of the Cochrane publication. *Source* Wong LF, Porter TF, Scott JR (2014) Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev (10):CD000112

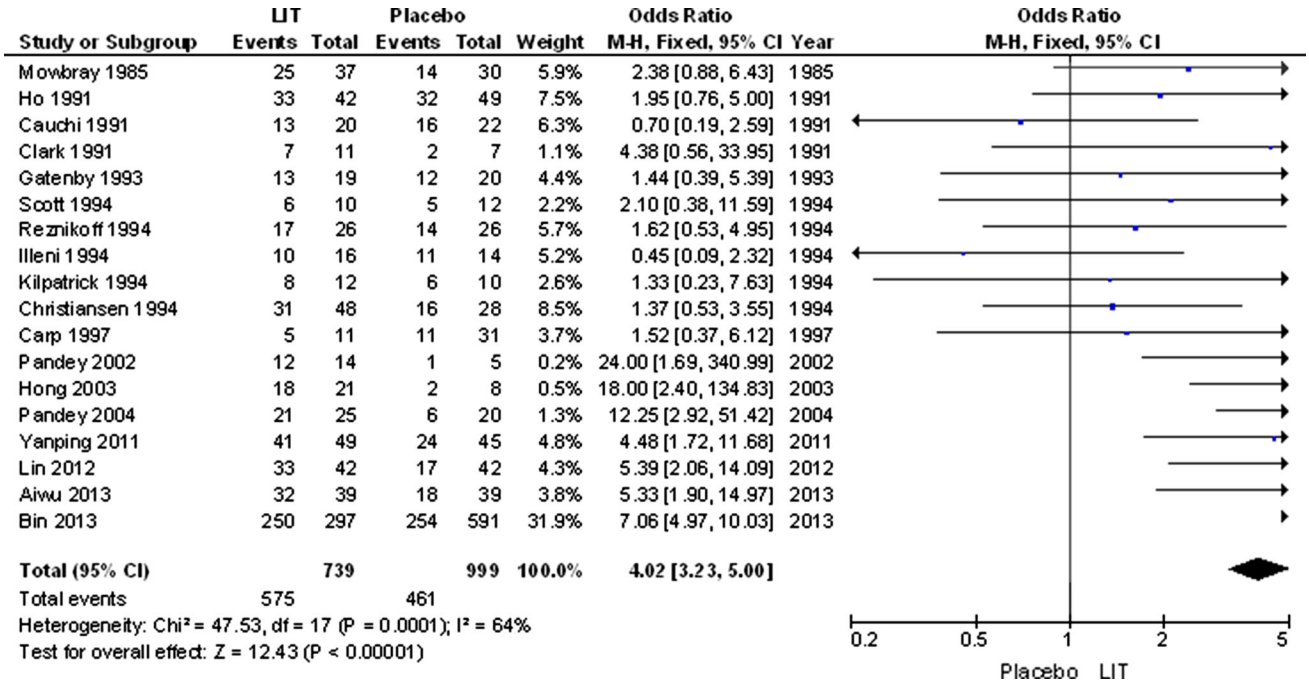

Fig. 2 Statistics of the meta-analysis by Liu et al. on the effect of immunotherapy with lymphocytes in cases of RM. *Source* Liu Z, Xu H, Kang X, Wang T, He L, Zhao A (2016) Allogenic lymphocyte immunotherapy for unexplained recurrent spontaneous abortion: a meta-analysis. Am J Reprod Immunol 76:443–453

Recently, to present the new evidence on the subject and correct the flaws of the Cochrane meta-analysis, Liu et al. published a new meta-analysis in the American Journal of Reproductive Immunology [27]. They included 18 randomized clinical trials, conducted from 1985 to 2013, for a total of 1738 patients, 739 in the group treated with immunization with paternal lymphocytes and/or unrelated donors and 999 in the control group [27]. Liu et al. demonstrated that immunization with lymphocytes promoted a significant improvement in the rate of live births: 77.8% in the group of treated women, when compared with the rate of 46.1% in the control group, with OR of 4.02 and CI of 3.23–5.00 (Fig. 2). In the meta-analysis of Liu et al., the data by Christiansen et al. are consistent with those presented in the original publication. Of all the studies included in the Cochrane meta-analysis [20], Liu et al. did

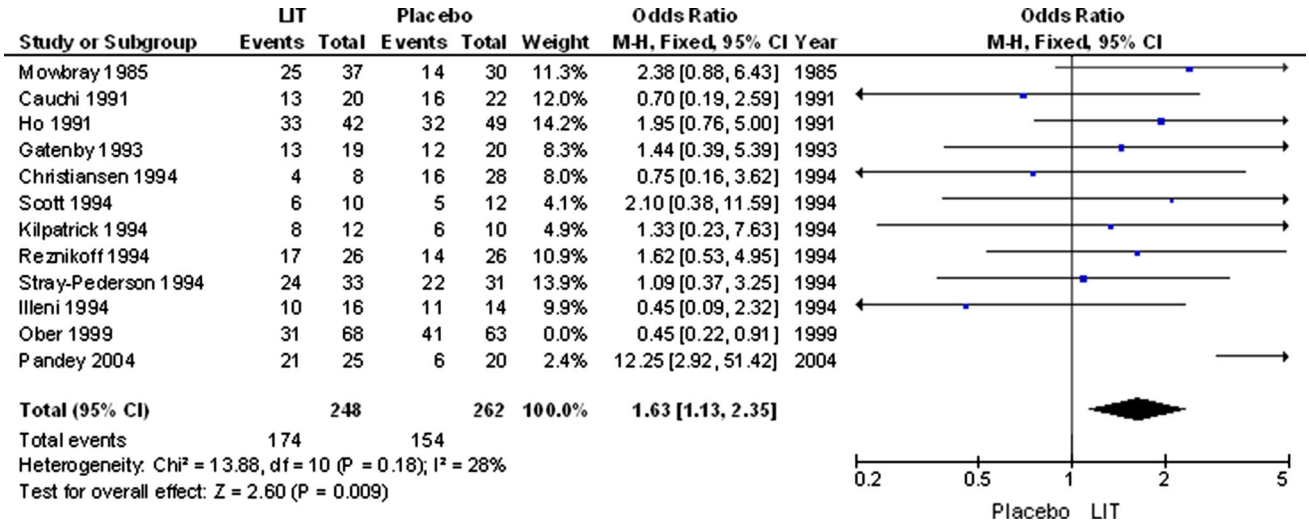

Fig. 3 Statistics from the Cochrane meta-analysis on the effect of immunotherapy with lymphocytes in cases of RM, removing the data by Ober et al. *Source* Ober C, Karrison T, Odem RB, Barnes RB, Branch DW, Stephenson MD (1999) Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomized trial. Lancet 354:365–369

not include data from Ober et al., given the methodological flaws that have been presented in the literature, nor the data by Stray-Pederson et al., as it did not appear in the authors' search base (Fig. 2) [27].

In a new analysis, including the data by Ober et al. [43] and by Stray-Pederson et al. (unpublished data), which were excluded by Liu et al., the improvement in the rate of live births in couples who underwent immunotherapy remained significant, with OR of 3.13 and CI of 2.56–3.82 (Test for overall effect: $Z = 20.11$, $P < 0.00001$). However, the inclusion of such data increased the heterogeneity of the sample (Heterogeneity: $\chi^2 = 84.23$, $df = 19$, $P < 0.00001$; $I^2 = 77\%$), being less homogeneous than the data observed in the original Cochrane and Liu et al. meta-analyses (Fig. 4).

Analyzing the data from Liu et al. separately, according to the source of lymphocytes for immunotherapy (whether paternal or unrelated donor), the effect of immunotherapy with lymphocytes on the reduction of miscarriage rates is still noticeable. The studies that used only paternal lymphocytes found OR of 2.45, with CI of 1.71–3.52 (Test for overall effect: $Z = 4.88$, $P < 0.00001$). That sample was slightly more homogeneous than that of the Cochrane meta-analysis (Heterogeneity: $\chi^2 = 20.74$, $df = 11$, $P = 0.04$; $I^2 = 47\%$) (Fig. 5).

Liu et al. performed statistical analyses on subgroups of patients, according to the protocols performed in clinical studies, contributing to the understanding of which protocol of immunization with lymphocytes presents the best results [27]. The first assessment was the period of the treatment, whether only before pregnancy or before and during pregnancy. Both forms of treatment significantly improved the rate of live births; however, when immunization with lymphocytes was performed before and during pregnancy, the results were better (OR 4.67, CI 3.70–5.90 versus OR 2.00, CI 1.39–2.88) [27]. Liu et al. also observed that the results were better when the concentration of lymphocytes used in the ILP was lower than 100×10^6 per application (OR 5.25, CI 4.16–6.64), when compared with a concentration greater than 100×10^6 per application (OR 1.52, CI 1.04–2.22) [27].

Studies with a lower level of evidence (case–control) suggest the need for laboratory criteria to identify couples who may benefit from immunotherapy with paternal lymphocytes, as well as the evaluation after immunotherapy, before allowing the couple to attempt a new pregnancy. Some of the suggested markers are as follows: (1) cross-match between the serum of patients and the spouse's lymphocytes to detect anti-lymphocyte maternal antibodies [45]; (2) mixed lymphocyte culture [46]; (3) assessment of the peripheral lymphocytes profile [47]; (4) assessment of Treg cells [16]; (5) assessment of interleukins Th-1 and Th-2 [14]; (6) assessment of NK cells [47]; and (7) soluble CD30 dose [48]. The pregnancy outcomes of couples who had a positive cross-match after immunotherapy were significantly better [25, 26, 45]. Recently, Yu et al. found that the effects caused by immunotherapy are best observed when the lymphocytes concentrate is administered intradermally, when compared with subcutaneous injection [45].

Safety of immunotherapy with lymphocytes

The first data regarding the safety of immunotherapy with lymphocytes were published in 1994 [25]. Maternal complications were observed in 2.1% (24/1149) of the women

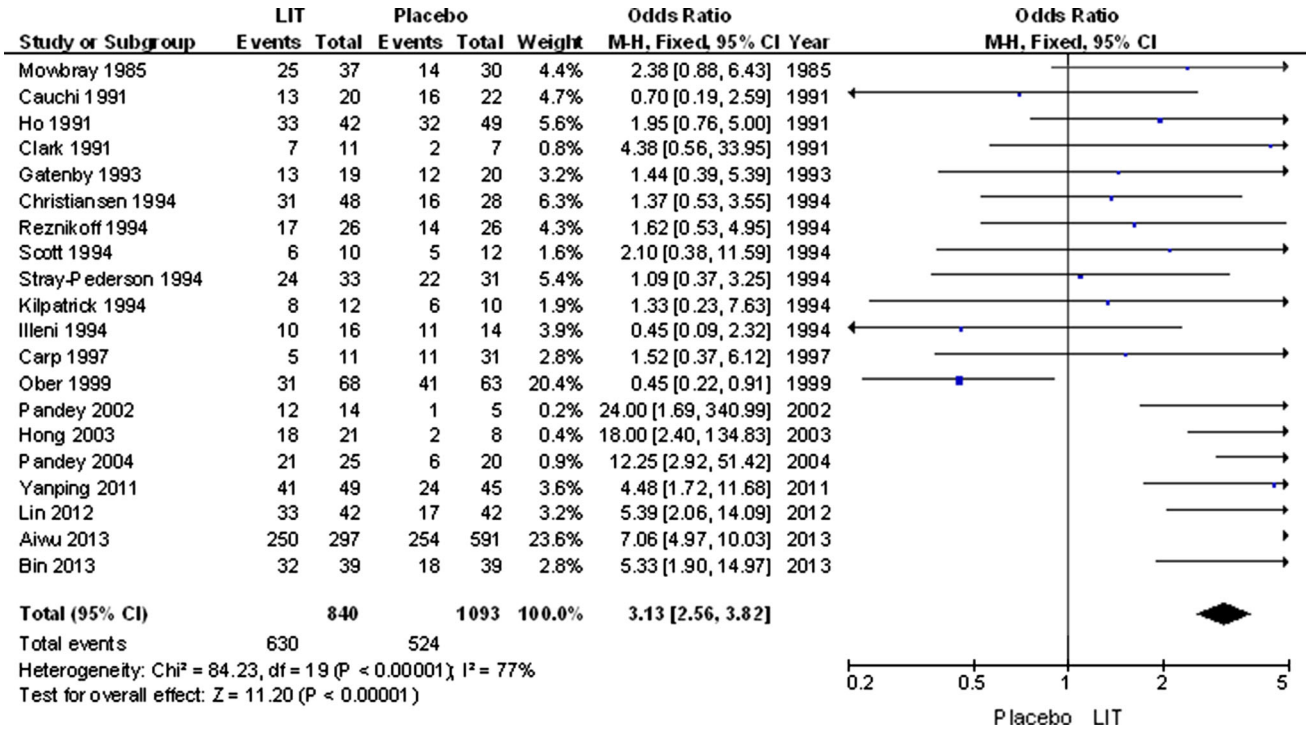

Fig. 4 Statistics including the results of the Cochrane and Liu et al. meta-analyses on the effect of immunotherapy with lymphocytes in cases of RM. *Source* Wong LF, Porter TF, Scott JR (2014) Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev (10):CD000112. Liu Z, Xu H, Kang X, Wang T, He L, Zhao A. Allogenic lymphocyte immunotherapy for unexplained recurrent spontaneous abortion: a meta-analysis. Am J Reprod Immunol 2016 76:443–453

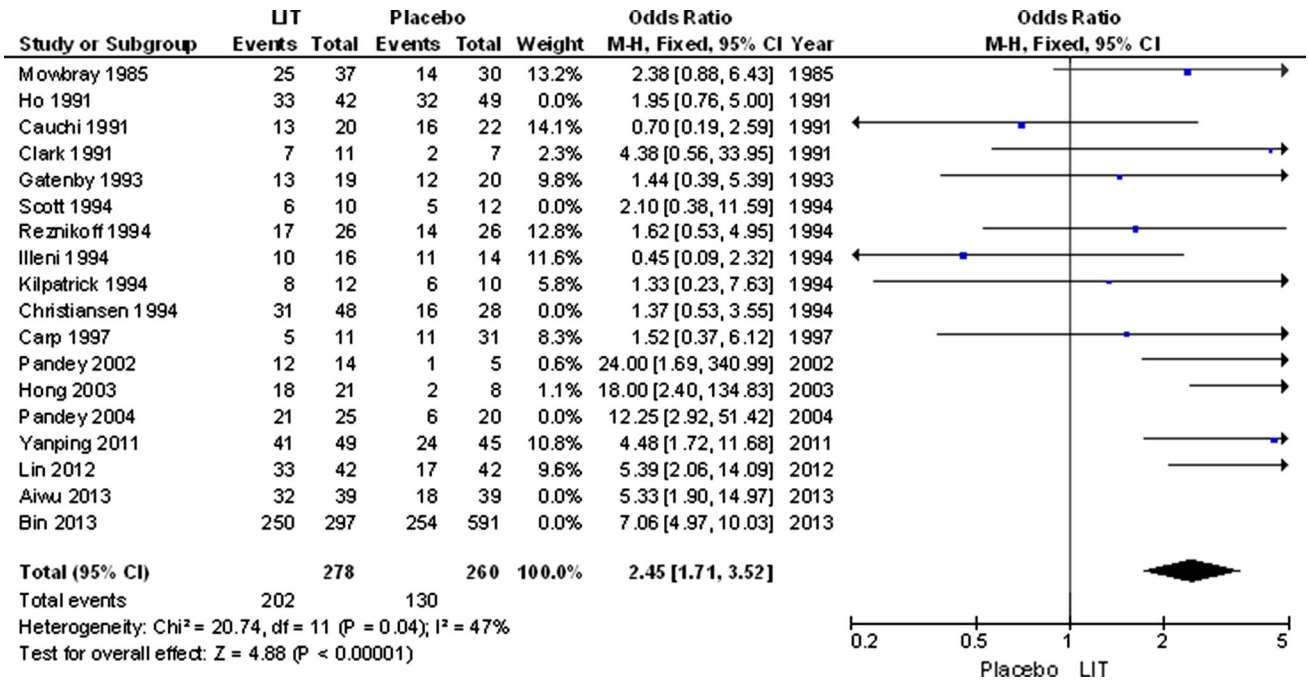

Fig. 5 Statistics from the Liu et al. meta-analysis on the effect of immunotherapy with lymphocytes in cases of RM, including studies that used only paternal lymphocytes. *Source* Liu Z, Xu H, Kang X, Wang T, He L, Zhao A (2016) Allogenic lymphocyte immunotherapy for unexplained recurrent spontaneous abortion: a meta-analysis. Am J Reprod Immunol 76:443–453

treated with immunization with lymphocytes, more frequently than in the control group, in which 0.5% of the women (2/410) were affected. The most commonly observed maternal complications were viral infections (hepatitis and cytomegalovirus), flu-like symptoms, and fever (transfusion reaction). Fetal (preterm birth, intrauterine growth restriction, fetal death, failure to thrive) and neonatal complications (neonatal thrombocytopenia and congenital malformations) were similar between both groups, 3% (36/1149) in the treated group and 4% (18/410) in the placebo group [25].

In 2006, Kling et al., who had experience in intradermal immunotherapy with lymphocytes since 1985, published a comprehensive study assessing the safety of immunotherapy. The study assessed the short- and long-term side effects in infertile couples or those with RM history who underwent immunotherapy with paternal lymphocytes. A retrospective evaluation of 3041 cases was performed, with a 2- to 3-year follow-up, in the period between 1996 and 2002; a prospective evaluation was performed between 2000 and 2003 [49].

The most frequently observed side effects were reactions at the application site of concentrated paternal lymphocytes, with a maximum duration of 15 days. Systemic reactions were reported by 8% ($n = 203/2587$) of patients [50]. Immunotherapy with paternal lymphocytes presents a theoretical risk of infectious diseases, which is controlled by performing regular partner serology. It is worth mentioning that, during routine intercourse, couples are at risk of transmitting the same diseases that are transmitted by blood donation. In the assessment by Kling et al., immunotherapy with paternal lymphocytes did not increase the frequency of other autoimmune complications, with a similar prevalence to that expected for the population not exposed to the immune treatment [49, 50].

Conclusion

In 2002, the Food and Drug Administration limited the use of immunotherapy with lymphocytes to research projects only [51], concerned about the safety of the treatment and the results presented by Ober et al. [43] in 1999, which showed a rise in miscarriage rates in women undergoing immunotherapy with lymphocytes. Since then, different centers worldwide have continued to perform immunotherapy with lymphocytes, improving immunological research and follow-up protocols for couples with the history of RM. Current evidence demonstrates that immunotherapy with lymphocytes appears to be a safe treatment. When performed with fresh, non-stored blood; before and during pregnancy; with an adequate concentration of lymphocytes; and applied intradermally to well-selected couples, it is a valid treatment for couples with history of RM of unknown cause.

Compliance with ethical standards

Funding This study was not funding.

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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